

CLAIMS

1. An isolated or purified strain of severe acute respiratory syndrome-associated human coronavirus,
5 characterized in that its genome has, in the form of complementary DNA, a serine codon at position 23220-23222 of the gene for the S protein or a glycine codon at position 25298-25300 of the gene for ORF3, and an alanine codon at position 7918-7920 of ORF1a or a
10 serine codon at position 26857-26859 of the gene for the M protein, said positions being indicated in terms of reference to the Genbank sequence AY274119.3.
2. The isolated or purified coronavirus strain as
15 claimed in claim 1, characterized in that the DNA equivalent of its genome has a sequence corresponding to the sequence SEQ ID NO: 1.
3. An isolated or purified polynucleotide,
20 characterized in that its sequence is that of the genome of the isolated coronavirus strain as claimed in claim 1 or claim 2.
4. The isolated or purified polynucleotide as claimed
25 in claim 3, characterized in that its sequence is SEQ ID NO: 1.
5. A pair of primers capable of amplifying a fragment of the sequence of the genome of a SARS-associated
30 coronavirus or of its DNA equivalent, characterized in that it is selected from the group consisting of:
 - the pair of primers No. 1 corresponding respectively to positions 28507 to 28522 (sense primer, SEQ ID NO: 60) and 28774 to 28759 (antisense primer,
35 SEQ ID NO: 61) of the sequence of the polynucleotide as claimed in claim 3 or claim 4,
 - the pair of primers No. 2 corresponding respectively to positions 28375 to 28390 (sense primer,

SEQ ID NO: 62) and 28702 to 28687 (antisense primer, SEQ ID NO: 63) of the sequence of the polynucleotide as claimed in claim 3 or claim 4, and

- 5 - the pair of primers consisting of the primers SEQ ID Nos: 55 and 56.

6. A probe capable of detecting the presence of the genome of a SARS-associated coronavirus or of a fragment thereof, characterized in that it is selected
10 from the group consisting of the fragments corresponding to the following positions of the polynucleotide sequence as claimed in claim 3 or claim 4: 28561 to 28586, 28588 to 28608, 28541 to 28563 and 28565 to 28589 (SEQ ID NO: 64 to 67).

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7. A recombinant cloning and/or expression vector, characterized in that it comprises an insert having the sequence SEQ ID NO: 38 and it is contained in a bacterial strain and it was deposited under the No.
20 I-3048, on June 5, 2003, at the Collection Nationale de Cultures de Microorganismes, 25 rue du Docteur Roux, 75724 Paris Cedex 15.

8. A recombinant cloning and/or expression vector,
25 characterized in that it contains a cDNA fragment selected from the group consisting of:

- a cDNA fragment encoding a C-terminal fusion of the N protein (SEQ ID NO: 37) with a polyhistidine tag, and
30 - a cDNA fragment encoding an N-terminal fusion of the N protein (SEQ ID NO: 37) with a polyhistidine tag.

9. The recombinant expression vector as claimed in claim 8, characterized in that it is contained in a bacterial strain which was deposited under the No.
35 I-3117, on October 23, 2003, at the Collection Nationale de Cultures de Microorganismes, 25 rue du Docteur Roux, 75724 Paris Cedex 15.

10. A cell modified with a vector as claimed in any one of claims 7 to 9.

11. A hybridoma producing a monoclonal antibody
5 against the N protein, characterized in that it is chosen from the following hybridomas:

- the hybridoma producing the monoclonal antibody 87, deposited at the CNCM on December 1, 2004 under the number I-3328,

10 - the hybridoma producing the monoclonal antibody 86, deposited at the CNCM on December 1, 2004 under the number I-3329,

- the hybridoma producing the monoclonal antibody 57, deposited at the CNCM on December 1, 2004 under the 15 number I-3330, and

- the hybridoma producing the monoclonal antibody 156, deposited at the CNCM on December 1, 2004 under the number I-3331.

20 12. A polyclonal or monoclonal antibody or antibody fragment directed against the N protein, characterized in that it is produced by a hybridoma as claimed in claim 11.

25 13. A chip or filter, characterized in that it comprises an antibody or an antibody fragment as claimed in claim 12.

30 14. An immunocapture test intended to detect a SARS-associated coronavirus infection, characterized in that it uses a monoclonal antibody specific for the native viral nucleoprotein (N protein).

35 15. The immunocapture test as claimed in claim 14, characterized in that the antibody used for the capture of the native viral nucleoprotein is a monoclonal antibody specific for the central region and/or for a conformational epitope.

16. The immunocapture test as claimed in claim 14 or
15, characterized in that the antibody used for the
capture of the N protein is the monoclonal antibody
mAb87, produced by the hybridoma deposited at the CNCM
5 on December 1, 2004 under the number I-3328.

17. The immunocapture test as claimed in claim 14 or
15, characterized in that the antibody used for the
capture of the N protein is the monoclonal antibody
10 mAb86, produced by the hybridoma deposited at the CNCM
on December 1, 2004 under the number I-3329.

18. The immunocapture test as claimed in claim 14 or
15, characterized in that the monoclonal antibodies
15 mAb86 and mAb87 are used for the capture of the
N protein.

19. The immunocapture test as claimed in any one of
claims 14 to 18, characterized in that the antibody
20 used for the visualization of the N protein is the
monoclonal antibody mAb57, produced by the hybridoma
deposited at the CNCM on December 1, 2004 under the
number I-3330, said antibody being conjugated with a
visualizing molecule or particle.

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20. The immunocapture test as claimed in any one of
claims 14 to 18, characterized in that a combination of
the mAb57 and mAb87 antibodies, conjugated with a
visualizing molecule or particle, is used for the
30 visualization of the N protein.

21. A reagent for the detection of a SARS-associated
coronavirus, characterized in that it is selected from
the group consisting of:

35 (a) a pair of primers as claimed in claim 5, or a
probe as claimed in claim 6,
(b) a recombinant vector as claimed in any one of
claims 7 to 9 or a modified cell as claimed in
claim 10,

- (c) an isolated coronavirus strain as claimed in claim 1 or claim 2 or a polynucleotide as claimed in either of claims 3 and 4,
5 (d) an antibody or an antibody fragment as claimed in claim 12,
(e) a combination of antibodies comprising the monoclonal antibodies mAb86 and/or mAb87, and the monoclonal antibody mAb57;
(f) a chip or a filter as claimed in claim 13.

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22. The use of a product selected from the group consisting of: a pair of primers as claimed in claim 5, a probe as claimed in claim 6, a recombinant vector as claimed in any one of claims 7 to 9, a modified cell as 15 claimed in claim 10, an isolated coronavirus strain as claimed in claim 1 or claim 2, a polynucleotide as claimed in claim 3 or claim 4, for the preparation of a reagent for the detection and optionally genotyping of a SARS-associated coronavirus.

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23. A method for the detection of a SARS-associated coronavirus, from a biological sample, which method is characterized in that it comprises at least:
(a) the extraction of nucleic acids present in said 25 biological sample,
(b) the amplification of a fragment of ORF-N by RT-PCR with the aid of a pair of primers as claimed in claim 5, and
(c) the detection, by any appropriate means, of the 30 amplification products obtained in (b).

24. The method as claimed in claim 23, characterized in that step (b) of detection is carried out with the aid of at least one probe corresponding to positions 35 28561 to 28586, 28588 to 28608, 28541 to 28563 and 28565 to 28589 of the sequence of the polynucleotide as claimed in claim 3 or claim 4.

25. A method for the detection of a SARS-associated

coronavirus infection, from a biological sample, by indirect IgG ELISA using the N protein, which method is characterized in that the plates are sensitized with an N protein solution at a concentration of between 0.5
5 and 4 µg/ml, preferably 2 µg/ml, in a 10 mM PBS buffer, pH 7.2, phenol red at 0.25 ml/l.

26. A method for the detection of a SARS-associated coronavirus infection, from a biological sample, by
10 double epitope ELISA, characterized in that the serum to be tested is mixed with the visualizing antigen, said mixture then being brought into contact with the antigen attached to a solid support.
- 15 27. An immune complex formed of a polyclonal or monoclonal antibody or antibody fragment as claimed in claim 11, and of a SARS-associated coronavirus protein or peptide.
- 20 28. A SARS-associated coronavirus detection kit or box, characterized in that it comprises at least one reagent selected from the group consisting of: a pair of primers as claimed in claim 5, a probe as claimed in claim 6, a recombinant vector as claimed in any one of
25 claims 7 to 9, a modified cell as claimed in claim 10, an isolated coronavirus strain as claimed in claim 1 or claim 2 and a polynucleotide as claimed in claim 3 or claim 4.
- 30 29. A fragment of the polynucleotide as claimed in claim 3, characterized in that it includes at least one pair of bases or pairs of bases corresponding to the following positions: 7919 and 23220, 7919 and 25298, 16622 and 23220, 19064 and 23220, 16622 and 25298,
35 19064 and 25298, 23220 and 24872, 23220 and 26857, 24872 and 25298, 25298 and 26857.